PB86-205614

Review of Literature on Chemical-Induced Aneuploidy in Mammalian Male Germ Cells

Oak Ridge National Lab., TN

Prepared for

Environmental Protection Agency
Washington, DC

1986

U.S. Department of Commerce
National Technical Information Service
NTIS

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before comple

1. REPORT NO. EPA/600/J-86/082	2. PB86-205614
4. TITLE AND SUBTITLE Review of Literature on Chemical-Induced Aneuploidy in Mammalian Male Germ Cells (Journal Version)	5. REPORT DATE 1986
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) James W. Allen, Jan C. Liang, Anthony V. Carrano, R. Julian Preston	8. PERFORMING ORGANIZATION REPORT NO. -
9. PERFORMING ORGANIZATION NAME AND ADDRESS Oak Ridge National Laboratory Env. Mutagen, Carcinogen & Teratogen Info. Program Oak Ridge, TN 37830	10. PROGRAM ELEMENT NO.
	11. CONTRACT/GRANT NO. IAG#DW899-30922 Contract#68-02-3839
12. SPONSORING AGENCY NAME AND ADDRESS Office of Health & Environmental Assessment (RD-689) Reproductive Effects Assessment Group U.S. Environmental Protection Agency Washington, D.C. 20460	13. TYPE OF REPORT AND PERIOD COVERED Final
	14. SPONSORING AGENCY CODE EPA/600/21

15. SUPPLEMENTARY NOTES
One of 12 articles prepared by a committee and including an introductory and summary paper. These reports were published together in Mutation Research, 167 (1986). Mutation Res. 167:123-137, January 1986

16. ABSTRACT

A committee of scientists was established by the U.S. Environmental Protection Agency to appraise the current state of aneuploidy test methodology, to compile and analyze published data on the chemical induction of aneuploidy, and to provide guidance for additional test development and validation. The committee was organized into 9 working groups, representing different eukaryotic organisms ranging from fungi to whole mammals. The Environmental Mutagen, Carcinogen, and Teratogen Information Program at Oak Ridge National Laboratory identified and provided literature to the working groups. The 9 reports were prepared by the individual working groups. The reports include: (1) descriptions of the available testing approaches and general principles to be followed for protocol development, (2) criteria for evaluating data, and (3) recommendations for future test development and basic research. The evaluated results are presented in tables. For the positive and negative calls, the lowest effective dose tested or the highest ineffective dose tested, respectively, and the range of concentrations tested are provided. An overall summary report was prepared that summarizes the conclusions and recommendations of the 9 working groups and the chemical data base. It is hoped that these reports will provide a framework for further test development and validation of aneuploidy assays.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT Release to the public	19. SECURITY CLASS (This Report) Unclassified	21. NO. OF PAGES 16
	20. SECURITY CLASS (This page) Unclassified	22. PRICE

EPA Form 2220-1 (9-73)

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

EPA/600/J-86/082
JOURNAL ARTICLE

Mutation Research, 167 (1986) 123–137
Elsevier

MTR 05010

Review of literature on chemical-induced aneuploidy in mammalian male germ cells *

James W. Allen [1], Jan C. Liang [2], Anthony V. Carrano [3] and R. Julian Preston [4]

[1] *Genetic Toxicology Division, Health Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, NC 27711,* [2] *Department of Genetics, University of Texas, M.D. Anderson Hospital and Tumor Institute, Houston, TX 77030,* [3] *Biomedical Sciences Division, Lawrence Livermore National Laboratory, Livermore, CA 94550, and* [4] *Biology Division, Oak Ridge National Laboratory, Oak Ridge, TN 37830 (U.S.A.)*

(Received 14 June 1985)
(Accepted 27 June 1985)

Contents

Summary 123
1. Introduction 124
2. The review process 124
3. Assay description 125
4. Interpretation of data 126
   4.1. Univalency 127
   4.2. Translocations 127
   4.3. Polyploidy 128
   4.4. Human YFF/Microtus sex chromosome body assays 128
5. Results and discussion 129
6. Conclusions 133
7. Recommendations 133
References 134

Summary

80 papers published between 1970 and 1984 were evaluated for results pertaining to chemical-induced aneuploidy in mammalian male germ cells. Diverse assays and end points were represented. The assays considered to involve direct measures of aneuploidy were based upon chromosome counts in premeiotic, meiotic, and embryonic cells, and the male pronucleus, or upon phenotypic expression of X-linked genetic markers. Assays in which indirect measures were interpreted as evidence for aneuploidy included those primarily assessing chiasma frequencies, univalent frequencies, and spermatid/sperm sex chromosome body counts.

* Report of the Aneuploidy Data Review Committee (Office of Research and Development, U.S. Environmental Protection Agency, Washington, D.C.)

The research described in this article has been reviewed by the Health Effects Research Laboratory, U.S. Environmental Protection Agency and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the Agency nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

This review has been funded by the U.S. Environmental Protection Agency through Contract No. 68-02-3839 and through an interagency agreement (No. DW899-30922) to the Oak Ridge National Laboratory.

Address reprint requests to the Environmental Mutagen, Carcinogen and Teratogen Information Program, P.O. Box Y, Oak Ridge National Laboratory, Oak Ridge, TN 37830, U.S.A.

0165-1110/86/$03.30 © 1986 Elsevier Science Publishers B.V. (Biomedical Division)

An initial screening to reject studies with insufficient data and those which did not involve a single chemical test agent led to the elimination of 39 papers from further review. The remaining 41 papers reported effects from 46 different chemicals. These papers were rigorously assessed for adequacy of experimental protocols, relevance of end points as direct measures of aneuploidy, and completeness of data presentation and statistical analysis. Criteria specific to each assay were also considered.

4 chemical tests were considered to provide reliable positive or negative aneuploidy data. Cyclophosphamide and chloral hydrate each caused metaphase II hyperploidy when injected into mice. Very limited analyses of trenimon and isoniazid provided negative results. Test findings for 44 chemicals were viewed as inconclusive. It was concluded that standardization of tests to evaluate chemical-induced aneuploidy in male germ cells and the application of these tests towards increasing the data base are badly needed.

(1) Introduction

Most of the human aneuploidy load is considered to be of germ cell origin. Approximately 5% of recognizable human pregnancies are cytogenetically abnormal. These abnormalities are primarily aneuploidies thought to stem from errors of meiotic chromosomal segregation (Hook, 1983; Bond and Chandley, 1983). In gametes, however, the true frequency of aneuploidy is uncertain. Estimates made from studies with males suggest that gametic aneuploidy may fall in the 1–5% range (Bond and Chandley, 1983). Segregational errors occurring in oogenesis are more frequent and thus of greater clinical significance than those occurring in spermatogenesis. Nevertheless, paternal contributions are also very important (e.g., in limited studies, they have been estimated to account for 6–15% of spontaneous abortions and 25% of liveborns with autosomal trisomy (Hansmann, 1983)). As nondisjunction of either maternal or paternal meiotic chromosomes clearly presents a health hazard, there is much concern that exposure of individuals during their reproductive years to environmental chemicals may induce germ cell aneuploidy and greatly enhance the degree of ill-health in their offspring.

In animals, spermatocyte and oocyte aneuploidy levels may increase with age and with exposure to temperature variations, radiation, and chemicals (Bond and Chandley, 1983; Hansmann, 1983; Sankaranarayanan, 1982). The molecular mechanisms involved are difficult to determine and, at present, remain poorly understood. In the case of irradiation, aneuploidy apparently arises, in large part, as a consequence of chromosome breakage and rearrangement. Some chemicals may induce aneuploidy as a secondary event to clastogenesis; other chemicals could act by pathways unrelated to DNA effects (e.g., chemical–protein interactions to alter the spindle or microtubules).

The literature is replete with information on diverse classes of chemicals that have been tested in various systems for the induction of aneuploidy. This committee report describes an effort to review the literature that specifically pertains to chemical-induced aneuploidy in mammalian male germ cells. Studies were assessed in terms of the results obtained and the validity of methods used. The aim is to determine which chemicals were reliably tested and found to be positive or negative for aneuploidy induction during spermatogenesis.

(2) The review process

A total of 80 papers were evaluated for evidence relevant to chemical-induced aneuploidy in mammalian male germ cells. Of these, 77 papers were located through the Aneuploidy File, Oak Ridge National Laboratory (described in introductory paper, Dellarco et al., this volume), and issued to the review committee. Three additional papers were included. The issued papers were only those published between 1970 and 1983. No effort was made to retrieve papers published earlier or those not listed in the Aneuploidy File. Thus, the present review reflects analyses of a restricted data base rather than a comprehensive assessment of all relevant literature.

The papers were first screened to determine if

they met certain standards of appropriateness for inclusion as chemical-induced aneuploidy data (Dellarco et al., 1985). Rejection criteria are listed below:

(1) Lack of relevant data — insufficient original aneuploidy data (e.g., abstracts, reviews).
(2) Lack of control data — insufficient generation or description of untreated and/or solvent control data.
(3) Other
    (a) foreign language;
    (b) incidental observations;
    (c) nonchemical agent;
    (d) exposure to a combination of agents;
    (e) unidentified agents.

Of the 80 papers screened for acceptability to review, 39 were rejected. 7 papers lacked relevant data in that they either did not pertain to aneuploidy or they were review articles with insufficient or no original data. One paper did not reveal sufficient information regarding control data. A total of 31 papers were rejected on the basis of the 'other' criteria (No. 3 above). Of these, two reported aneuploidy observations that were incidental to the measure of other end points, with little or no data to support claims. In 27 instances, the agents involved were not chemical; they were concerned with age, temperature, virus, or radiation, or there was no test agent or an unidentified one. Two other papers dealt with effects from exposure to a combination of agents.

The 41 papers remaining after the initial screen were reviewed in detail for evidence of chemical-induced aneuploidy during male germ cell development.

(3) Assay description

Most germ cell aneuploidy investigations in the male mammal are carried out with experimental rodent systems in which animals are treated with test agents by various routes of exposure. A few studies of human spermatozoa have been conducted, usually with samples from untreated individuals. These systems incorporate a variety of assay approaches and end points that are used to study aneuploidy or presumed aneuploidy-related events.

Table 1 shows the stages of spermatogenesis and post-fertilization development at which evidence for aneuploidy has been evaluated. Spermatogonial cells undergo a series of mitotic divisions prior to becoming meiocytes. Chromosomes may be counted in metaphase cells to reveal evidence of mitotic nondisjunction. Prophase and metaphase (MI) primary spermatocyte stages are analyzed, respectively, for chiasma frequencies and the incidence of univalents. These are questionable measures of aneuploidy, and papers reporting only these end points were considered inconclusive (Section 4.1). Should maternal and paternal homologues fail to synapse properly, and/or to cross-over, fewer chiasmata and more univalents per cell should result. Secondary spermatocyte metaphases may reveal missing or extra chromosomes if univalent (or bivalent) chromosomes fail to segregate properly at anaphase I. Aneuploidy can also result from the segregation of translocations during meiosis (Section 4.2).

A specific assay utilizing the field vole, *Microtus oeconomus*, takes advantage of sex chromosome heterochromatin patterns that are uniquely identifiable in spermatids stained with C-banding techniques (Tates et al., 1975). Extra sex bodies in spermatids have been used as indicators of sex-chromosome nondisjunction. Similarly, part of the human Y chromosome fluoresces brightly with quinacrine dihydrochloride; two fluorescent bodies (YFF) in sperm are presumed to result from Y chromosome nondisjunction (Pawlowitzki and Pearson, 1972). Both of these methods are questionable as indicators of aneuploidy (Section 4.4), and all papers reporting only data on these end points were considered inconclusive. A relatively new method for analyzing human spermatozoa chromosome complements relies on in vitro fertilization of Syrian hamster eggs and decondensation of the sperm chromatin (Rudak et al., 1978; Martin et al., 1983). Chromosome counts in the male pronucleus provide an estimate of germ line non-disjunction events for individual chromosomes. Animal models (rodent sperm) have also been reported (Yanagimachi, 1982). Thus far, only spontaneous aneuploidy levels have been studied, and no papers on chemical effects were available for consideration.

Embryos from first cleavage to mid-gestation stages have been scored for chromosome numbers.

TABLE 1

ASSAYS USED TO EVALUATE ANEUPLOIDY IN MALE GERM CELLS

Cells or organisms analyzed	Measures of aneuploidy		Origins of aneuploidy
	Direct	Indirect or questionable	
Spermatogonia	Chromosome counts at metaphase		Mitotic nondisjunction
Primary spermatocytes		Chiasma frequencies at prophase; univalent frequencies at metaphase I	Nonconjunction (failure of homologue pairing or crossing-over)
Secondary spermatocytes	Chromosome counts at metaphase II		Missegregation of univalents; nondisjunction of homologues
Spermatids		X, Y bodies	Meiotic nondisjunction
Spermatozoa			
Prefertilization		YFF bodies	Meiotic nondisjunction
Postfertilization	Chromosome counts in male pronucleus		Meiotic nondisjunction
Embryo cells	Chromosome counts at metaphase		Meiotic or postmeiotic damage; postfertilization nondisjunction
F_1 adults	Phenotypic expression of X-linked genetic markers; chromosome counts		Meiotic nondisjunction or postmeiotic damage; postfertilization nondisjunction

These studies usually are timed such that conceptuses would have been derived from sperm subjected to chemical insult during postmeiotic development. Evidence for aneuploidy detected beyond the first cleavage is indirect since that arising in germ cells cannot be distinguished from that arising in the embryo. Finally, a genetic analysis of aneuploidy may be conducted with the numerical sex-chromosome anomaly method (Russell, 1976). After paternal treatment with the test chemical, F_1 mice are evaluated either for chromosome counts or for phenotypes reflecting aneuploidy for X-linked genetic markers. This assay will only detect those nondisjunction events that are transmitted to the F_1.

(4) Interpretation of data

Although the assays used to detect male germ line aneuploidy are heterogeneous, it was possible to evaluate them with common criteria together with specific considerations for each assay. The common criteria included general adequacy of the experimental protocols, relevance of the end point as a direct measure of aneuploidy, and the completeness of data presentation and statistical analysis.

A compound could be considered either positive or negative for aneuploidy induction if: (a) Experimental protocol was acceptable in terms of control groups, sample sizes, and other critical aspects of experimental design. (b) Reporting of aneuploidy findings was clear (i.e., hyperploidy distinguished from hypoploidy and polyploidy). Only direct measures of aneuploidy were acceptable, and statistical analyses of hyperploidy were required for a positive or negative response. (c) Metaphase chromosomal complements revealed significantly increased frequencies of hyperploid cells. Hypoploidy alone theoretically may provide a true index of aneuploidy (Ford and Roberts, 1983; Cattanach et al., 1984; Russell, 1976). It is

generally unreliable as a single end point due to the potential for confusion with artifacts resulting from slide preparation. For this reason, papers reporting only hypoploidy were regarded as inconclusive.

Inconclusive results were determined by the following criteria:

(1) Protocol uncertainties — Related to dose, mode of exposure, cell-type treated, exposure time, controls, or scoring criteria. Negative results based on only one dose and one sampling time were considered to be inconclusive.
(2) Indirect or questionable criteria
   (a) univalency;
   (b) translocations;
   (c) polyploidy;
   (d) human YFF or Microtus sex chromosome bodies.
(3) Equivocal results
   (a) hypoploidy only appearing questionably artifactual;
   (b) different ploidies combined/no separate hyperploidy data reported;
   (c) small sample size;
   (d) uncertain statistical significance.

The protocol uncertainties and equivocal results sections are self-explanatory and will not be discussed further. Some explanation is warranted on the indirect or questionable criteria.

(4.1) Univalency

In addition to the usual mechanisms of nondisjunction proposed for mitotic chromosomes, primary spermatocyte chromosomes may fail to distribute properly in anaphase I because of abnormal homologue synapsis or desynapsis. Homologues that do not pair or synapse are expected to complete meiotic prophase and metaphase I as unassociated univalents. Alternatively, bivalents in which there is a failure to cross-over and establish a chiasma between homologues or in which there is an early loss of this association theoretically can give rise to univalents. In these circumstances two homologous univalents would be expected, and chromosome numbers per cell would be normal. However, single univalents in hypoploid or hyperploid primary spermatocytes can also occur. These conditions could arise from premeiotic nondisjunction, and univalency of this type can be viewed as evidence of aneuploidy.

Several studies have reported a decrease in total numbers of chiasmata, an increase in terminal chiasmata, and an increase in univalency in old female mice (Henderson and Edwards, 1968) and hamsters (Sugawara and Mikamo, 1983). Chiasmata serve to hold repelling maternal and paternal homologues together until anaphase I, and thus mediate normal homologue segregation. Without chiasmata, homologues may segregate randomly, or in nonrandom ways (Grell, 1959), resulting in aneuploid secondary spermatocytes.

Although a clear rationale exists for interpreting univalency as a portent of anaphase I nondisjunction and metaphase II (MII) aneuploidy, there is little evidence to substantiate this relationship. In one study using a marker bivalent in female mice (De Boer and Van Der Hoevern, 1980), increases is univalency and nondisjunction (MII aneuploidy) as a result of aging did appear to correlate. In other studies involving female mice (Polani and Jagiello, 1976; Speed, 1977) and Chinese hamsters (Sugawara and Mikamo, 1983), no correlation between univalency incidence and nondisjunction was found. The latter study revealed that univalency and MII aneuploidy did indeed increase with age. These increases were reflected in different chromosome groups, thereby dispelling any notion that the events were related.

In chemical test trials, the reliability of univalency measurements is questionable. Normal untreated animals reveal highly variable incidences of univalency between species, strains, and individuals (Gollapudi et al., 1981). Sex bivalents and small autosomal bivalents with single, relatively terminal chiasmata may be more vulnerable to stretching apart during slide preparation procedures. It is speculated that much of the observed univalency actually may be artifactual (Polani and Jagiello, 1976). In view of the highly questionable nature and fate of univalents, at this time little significance should be attached to this criterion as a meaningful indicator of aneuploidy.

(4.2) Translocations

Chromosomal translocations may lead to aneuploidy induction during meiotic division. For example, translocations forming quadrivalents, such as R IV and C IV, may undergo 3 : 1 segrega-

tion at anaphase I, resulting in the production of aneuploid gametes. Other segregation errors (e.g., the adjacent II) may also occur, producing pseudohaploid gametes in which the total chromosome number remains the same, but duplications or deficiencies of individual chromosomes may result. Such chromosomal changes usually are not detectable without banding analysis. Since the frequency of translocation-derived aneuploidy may vary, depending on the specific chromosomes involved in the translocation and the types of translocations, it cannot be predicted by MI analysis. Such a frequency can only be determined in MII, a stage subsequent to anaphase I in which chromosome numbers can be accurately counted. Although translocation in MI may be indicative of the potential for aneuploidy production in subsequent meiotic divisions, it should not serve as direct evidence of aneuploidy induction.

(4.3) Polyploidy

There is no evidence at the present time for any mechanistic relationship between polyploidy and aneuploidy. Therefore, measures of polyploidy were not interpreted here to infer aneuploidy.

(4.4) Human YFF / Microtus sex chromosome body assays

The Microtus sex chromosome and the human YFF assays have been used as a measure of aneuploidy in their respective biological systems. They are discussed together here because they are both dependent on cytochemical methods for the analysis and both end points are measured in spermatids or mature sperm. At the present time, however, the methods lack genetic validation, and we have chosen not to view them as direct measures of aneuploidy.

The Microtus sex chromosome assay is based on the observation that both the X and Y chromosomes of *Microtus oeconomus* have large heterochromatic regions and stain intensely with a C-banding procedure (Tates et al., 1975). In interphase cells, spermatids, and mature sperm, the heterochromatic regions stain heavily with Giemsa. The assumption is that these cytochemically distinct regions represent the sex chromosomes. In early spermatids, where the assay appears to be ambiguous, the Y chromosome is believed to be observed as a distinct dark-staining compact granule, while the X chromosome is thought to be the larger, more diffuse spot. Initial studies indicated that the incidence of XX, XY, and YY spermatids was increased by exposure of the animal to X-rays and several chemicals (Tates, 1979; Tates et al., 1979). In a later study, the increased incidence of aneuploidy resulting from X-ray exposure could not be confirmed (Tates and de Vogel, 1981). The authors suggested several possibilities for this lack of reproducibility, including genetic selection in the Microtus breeding colony against animals with a sensitivity toward nondisjunction. Because of this lack of reproducibility, the assay cannot yet be considered a reliable indicator of aneuploidy.

The cytochemical basis of the human YFF assay derives from the presence of heterochromatin in the distal portion of the long arm of the Y chromosome. This region is enriched in repetitive DNA and fluoresces brightly in metaphase chromosomes, interphase cells, and sperm when stained with the fluorochrome quinacrine dihydrochloride (Caspersson et al., 1970; Barlow and Vasa, 1970). The presence of a single bright fluorescent spot in mature sperm suggests the presence of a single Y chromosome, two spots suggest the presence of two Y chromsomes, and the absence of a bright spot suggests that the sperm bears an X chromosome. As with the Microtus assay, sample preparation is simple and the analysis is rapid, but the method is without proper genetic validation. Studies have shown that the frequency of two bright spots in the sperm of control men is about 1% (Pawlowitzki and Pearson, 1972; Kapp et al., 1979; Kapp and Jacobson, 1980; Klasen and Schmid, 1981). If this frequency represents the true nondisjunction frequency of the Y chromosome and is used to estimate aneuploidy for all the chromosomes, the total frequency would be very high (about 40%). More recent techniques that allow direct evaluation of chromosomes in human sperm, following in vitro fertilization of Syrian hamster embryos, do not support the high Y chromosome aneuploidy frequencies found with the YFF method (Rudak et al., 1978; Martin et al., 1983; Brandriff et al., 1984; Wyrobek et al., 1984). The bright regions observed with the YFF assay may reflect the quinacrine-bright regions of other chromosomes or may result from separation of the Y

heterochr
this tech
indicator

omatin in sperm. Whatever the cause, nique cannot be accepted as a reliable of human aneuploidy.

(5) Resul

ts and discussion

41 art
chemical
at least t
59 chem
measures
ing studi
ploidy, X
tions. No
with cher
genetic (r
or in vitr
proaches.

Only 4
ing reliab

icles reporting effects from 46 different s were reviewed. 9 chemicals were tested wice in separate studies, giving a total of ical test trials. In 23 instances, direct of aneuploidy were applied. The remain- es were concerned with univalency, poly- and/or Y bodies, and structural aberra- ne of the papers reviewed were concerned nical-induced aneuploidy analyses by the umerical sex chromosome anomaly test) o fertilization/sperm decondensation ap-

chemical tests were regarded as provid- le positive or negative aneuploidy results for the described experimental conditions (Table 2). Cyclophosphamide and chloral hydrate each caused statistically significant increases in metaphase II hyperploidy when injected i.p. into mice at various times during spermatogonial through metaphase I stages of development. For both chemicals, a simple dose–response relationship could not be demonstrated, presumably because of cell-lethal effects at the higher doses. Both chemicals also were clearly clastogenic, as evidenced by structural damage in metaphase II cells.

Trenimon, over a narrow test-dose range, was determined to be negative for aneuploidy induction in Chinese hamster spermatids. Analyses were carried out on 4-8-cell embryo stages when structural aberrations were significantly elevated. This chemical has been reported to be positive in some studies of aneuploidy induction in female rodent germ cells (Hansmann, 1983). It should be emphasized that negative findings reported here per-

TABLE 2

CHEMICA
TIONS AN

LS CONSIDERED POSITIVE OR NEGATIVE FOR ANEUPLOIDY INDUCTION: EXPERIMENTAL CONDI- D RESULTS

	Chemical			
	Cyclophosphamide (50-18-0)	Chloral hydrate (302-17-0)	Trenimon (2,3,5-Tris-ethylene-iminobenzo-1,4-quinone) (68-76-8)	Isoniazid (INH) (54-85-3)
Aneuploidy results	+	+	–	–
Species	mouse	mouse	Chinese hamster	Chinese hamster
Route of exposure	i.p.	i.p.	i.p.	Oral intubation
Cells exposed	Spermatogonia–metaphase I	Staminal gonia-pachytene	Spermatid	Primary spermato-cyte
Cells analyzed	Metaphase II	Metaphase II	4–8-cell embryo	Metaphase II
Dose range (mg/kg)	30–145	82.7–413.5	0.19 or 0.25	2 doses 24 h apart 5–125 mg/kg
LEDT/HIDT [a] (mg/kg)	30	82.7	0.25	2×125
Highest % hyperploidy (control)	4.0 ($\overline{0.9}$)	6.0 ($\overline{1.1}$)	0.9 ($\overline{0.9}$ historical)	–
Highest statistical difference from control	$P < 0.005$	$P < 0.005$	–	–
Dose response	No (only low dose effective)	Complex	–	–
Other effects	Fragments	Fragments	Structural aberrations	None
Citation	Pacchierotti et al., 1983	Russo et al., 1984	Binkert and Schmid, 1977	Adler et al., 1978

[a] LEDT, lowest effective dose tested; HIDT, highest ineffective dose tested.

TABLE 3

CHEMICALS CONSIDERED INCONCLUSIVE FOR ANEUPLOIDY INDUCTION

Chemical (CAS Registry No.)	Species [a]	Cell type [b] analyzed	Dose range [c] (mg/kg, except where indicated)	Inconclusive codes [d]	Reference
Acrylamide (79-06-1)	Mouse	Spermatogonia	100	1, 3b	Shiraishi, 1978
		MI	50–100 mg/ml and 500 ppm	2a * b *	
Adriamycin (23214-92-8)	Mouse	MI	3–24	2a *b *	Au and Hsu, 1980
Alcohol (64-17-5)	Mouse	Spermatogonia	14–136 g/kg	3a	Alvarez, 1983
Benomyl (17804-35-2)	Microtus	Spermatid	50–500	1, 2cd	Tates, 1979
Bleomycin (11056-06-7)	Microtus	Spermatid	0.05–0.5	1, 2cd	Tates, 1979
	Mouse	MI	10–40	2a *b *, 3a	Van Buul and Goudzwaard, 1980
Busulfan (55-98-1)	Human	Spermatogonia	20–80	2b	Kapp and Jacobson, 1980
		Sperm	N.D.	1, 2d	
Cadmium chloride (10108-64-2)	Rat	MI, MII	5–10 μmoles/kg	2a *c	Fowler et al., 1982
Cannabinol (521-35-7)	Mouse	MI	50 (×5)	1, 2ab *c	Dalterio et al., 1982
Carbaryl (63-25-2)	Human	Sperm	N.D.	1, 2d	Wyrobek et al., 1981
4-Chloromethylbiphenyl (1667-11-4)	Mouse	MII	100	1, 3a	Brook, 1982
Colcemid (477-30-5)	Microtus	Spermatid	3.7	1, 2cd	Tates, 1979
Cyclophosphamide (50-18-0)	Mouse	MI	40–160	2a *b *	Goetz et al., 1980a, b
		MI of F_1	80	2b *	
1,2-Dibromo-3-chloropropane (96-12-8)	Human	Sperm	1 ppm	1, 2d	Kapp et al., 1979
	Human	Sperm	1 ppm (×6–15 months)	1, 2d	Kapp and Jacobson, 1980
Ethyl methanesulfonate (62-50-0)	Mouse	MII	240	1	Szemere and Marczinovits, 1977
	Mouse	MII	240	3d	Szemere, 1978
4-fluoro-DL-β-phenylalanine (60-17-3)	Microtus	Spermatid	150 (×2)	1, 2cd	Tates, 1979
Formaldehyde (50-00-0)	Mouse	MI	50	2abc	Fontignie-Houbrechts, 1981

TABLE 3 (continued)

Chemical (CAS Registry No.)	Species [a]	Cell type [b] analyzed	Dose range [c] (mg/kg, except where indicated)	Inconclusive codes [d]	Reference
Frusemide (54-31-9)	Mouse	MI	0.0078–1.25 mg/ml	2a *b *c	Subramanyam and Jameela, 1977
Halothane (151-67-7)	Microtus	Spermatid	0.02%	1, 2cd	Tates, 1979
Isoniazid (54-85-3)	C. hamster	spermatogonia	5–125	3b	Miltenburger et al., 1978
Lysergic acid diethylamide (50-37-3)	Mouse	MI	0.5–2.0 μg/kg	2a *b *c, 3b	Mitra et al., 1979
	Rat	MI	10–1000 μg/kg	1, 2a *	Goetz et al., 1974
Malathion (121-75-5)	Mouse	MI	0.5 ml of 0.3% sol'n (×50–100)	1, 2a *c, 3b	Bulsiewicz et al., 1976
6-Mercaptopurine (50-44-2)	Microtus	Spermatid	75	1, 2cd	Tates, 1979
Methadone (76-99-3)	Mouse	MI	1–6	2a *b *c	Badr et al., 1979
Methoxychlor (72-43-5)	Mouse	MI	0.5 ml of 0.1% sol'n (×50–100)	1, 2a *c, 3b	Bulsiewicz et al., 1976
2-Methoxy-ethyl-mercury-chloride (123-98-6)	Mouse	MII	13	3d	Szemere, 1978
Methyl-2-benzimidazole-carbamate (10605-21-7)	Microtus	Spermatid	250 (×2)	1, 2cd	Tates, 1979
Methyl methane-sulfonate (66-27-3)	Microtus	Spermatid	50	1, 2cd	Tates, 1979
	Mouse	MII	50	1	Szemere and Marczinovits 1977
	Mouse	MII	50	3d	Szemere, 1978
	Mouse	Fertilized eggs	100	1, 2bc, 3d	Albanese, 1982
Mitomycin C (50-07-7)	Monkey	MI	1	1, 2a *b *, 3c	Ficsor et al., 1979
	Mouse	Embryo	2	1, 2c, 3ad	Chandley and Speed, 1979
	Mouse	MI	0.1–1.0 (×5)	2ab *	Adler, 1982
Nitrosan (2980-64-5)	Mouse	MI	10	1, 2a *	Nehez et al., 1978
Nocodazol (31430-18-9)	Microtus	Spermatid	120	1, 2cd	Tates, 1979
Oil of nutmeg (8008-45-5)	Mouse	MI	60–400 (×40)	2b	Pecevski et al., 1981
		MI of F_1	60–400 (×40)	2a *b *	
Pheniramine maleate (132-20-7)	Mouse	MI	0.75–2.24	2a *b *c	Subramanyam et al., 1981

TABLE 3 (continued)

Chemical (CAS Registry No.)	Species [a]	Cell type [b] analyzed	Dose range [c] (mg/kg, except where indicated)	Inconclusive codes [d]	Reference
Phenobarbital (50-06-6)	Mouse	MI	42–126	2a *b *c	Nandan and Rao, 1982
Phenol (108-95-2)	Mouse	Spermatogonia	0.08–8 mg/l (×daily 6 generations)	1, 2c, 3b	Bulsiewicz, 1977
		MI		1, 2a *c, 3b	
Procarbazine (366-70-1)	Microtus	Spermatid	100–400	1, 2cd	Tates, 1979
	Mouse	MI	50–200 (×4)	2ab *	Adler, 1982
Sodium selenite (10102-18-8)	Mouse	MI	0.8	1, 2ab	Norppa et al., 1980
Sulfur (7704-34-9)	Guinea pig	Spermatogonia	100 mg (×60–120)	1, 2c, 3b	Bulsiewicz and Wesolowski, 1977
		MI		1, 2a *c	
Δ-Tetrahydro-cannabinol (1972-08-3)	Mouse	MI	50 (×5)	1, 2a *b *c	Dalterio et al., 1982
Thiabendazole (148-79-8)	Mouse	MII	210 (×9–22)	1	Pachierotti et al., 1982
ThioTEPA (52-24-4)	Mouse	MI	1.66–5.0	2a *b *c	Devi and Reddy, 1980
Triaziquone (68-76-8)	C. hamster	Embryo	N.D.	1, 2b *	Schmid, 1977
Trichlorfon (52-68-6)	Mouse	MI	0.3% sol'n (ad libitum×50–100)	1, 2a *c, 3b	Bulsiewicz et al., 1976
Vincristine (54-22-7)	Microtus	Spermatid	0.05–0.2	1, 2cd	Tates, 1979
Vitamin C (50-81-7)	Mouse	MI	10 (×60)	1, 2abc, 3b	Pichan and Polasa, 1982

[a] Species: C. hamster, Chinese hamster; monkey, Rhesus monkey; Microtus, *Microtus oeconomus*.
[b] MI, first meiotic metaphase; MII, second meiotic metaphase.
[c] (×---), multiple dosing for number of times or over duration specified; N.D., no data.
[d] 1, protocol uncertainties; 2, indirect or questionable criteria: a, univalency, b, translocations, c, polyploidy, d, human YFF or Microtus sex chromosome bodies; 3, equivocal results, a, hypoploidy only, b, different ploidies combined/no separate hyperploidy data, c, small sample size, d, uncertain statistical significance. * Significantly elevated levels of univalents or translocations were observed.

tain only to evidence from embryo cells and thus are of limited value. Trenimon may have produced aneuploid cells that did not survive to embryo stages. Acute and chronic oral intubation exposures of Chinese hamsters to isoniazid also did not cause significant increases in aneuploidy. Although these two chemicals were considered negative, their analyses were limited either to a single dose or to a single cell stage. Definitive evidence of these negative results requires further testing.

Chemicals considered inconclusive for aneuploidy induction are listed in Table 3. In view of the diversity of test protocols used, no effort was made to resolve different test results for the same

chemical. In a total of 59 separate tests, 37 were determined to have protocol uncertainties. Univalency, translocations, and polyploidy were represented as primary end points in about half of the studies. When these end points were analyzed in combination with one another, more often than not they would correlate for general reactivity or nonreactivity. However, their proportionalities of increase and dose–response patterns were often dissimilar. Particularly high incidences of univalency (9–12 × baseline) were reported for some doses of adriamycin, cadmium chloride, and LSD. Although univalency was not analyzed in the study for which cyclophosphamide was concluded to be positive for aneuploidy induction, this end point was increased to 6 × baseline in a separate cyclosphosphamide study (Table 3). None of the studies indicated whether the univalents ever occurred as single events; thus, it is assumed that chromosome numbers per cell were normal. The univalents also were seldom defined for the extent of their dissociation from homologues. An asterisk was used to note a positive response (for any of the end points) as interpreted by the author or the reviewer. The chemicals so noted may be considered candidates for further testing using direct measures of aneuploidy.

25% of the inconclusive studies were YFF or Microtus sex chromosome body analyses. Equivocal results, primarily due to scoring or reporting habits in which hyperploidy either was not analyzed or not discernible from combined ploidy data, characterized another 27% of the inconclusives.

(6) Conclusions

A number of conclusions can be drawn from this review of the published literature on the induction of aneuploidy by chemicals in mammalian male germ cells. Several of these conclusions are discussed further as recommendations in Section 7.

(a) Few published reports provide an adequate test for the induction of true aneuploidy in male germ cells. The reasons for the inadequacy of many of the tests are discussed in Section 4.

(b) Standard assays have not been developed or utilized. While an invariable protocol is not necessary, certain general principles should guide the development of assays. These are discussed for analyses of metaphase II and first cleavage divisions (Section 7).

(c) The majority of papers reviewed could not be considered as reporting adequate data for determining the induction, or lack of induction, of true aneuploidy in male germ cells. In some cases the protocol itself was inadequate or led to equivocal results (Section 4). Data were often reported in such a way that a measure of true aneuploidy as opposed to technical artifacts could not be ascertained, due to the combining of the frequencies of hyperploidy and hypoploidy. In addition, certain of the end points (e.g., univalency, polyploidy, YFF) have not clearly been shown to be directly related to aneuploidy and could not be considered as appropriate.

(d) Chloral hydrate and cyclophosphamide were sufficiently well tested to allow the conclusion that they were inducers of aneuploidy, when analysis was performed on metaphase II cells. Trenimon and isoniazid were considered noninducers of aneuploidy, although the tests on which this conclusion was based were less complete than those for the positive compounds (for discussion, see Section 5).

(7) Recommendations

A detailed consideration of the published mammalian literature on the induction of aneuploidy following chemical treatment of male germ cells indicates that considerably more data are needed and that more standardized approaches to experimental design, data collection, and reporting are essential.

It is not appropriate to recommend a complete protocol to be followed step by step. Certain general principles should be considered and adhered to whenever appropriate and possible. Many cell stages of spermatogenesis are amenable to treatment, but it is perhaps most informative and least equivocal to analyze for aneuploidy at MII in spermatocytes or at the first cleavage division following fertilization. In both cases, the treated cells are premeiotic or early meiotic (prior to metaphase I). The time between treatment and observation is used to determine the treated cell type.

It is strongly recommended that studies should employ a sufficient number of dose levels to test for a dose–response effect (i.e., a minimum of 3 doses and a solvent or vehicle control). The maximum dose should be one that induces a significant level of toxicity (e.g., inhibition of spermatogenesis or mortality). The other doses should span at least an order of magnitude below this maximum dose.

The methods of cell preparation should be those that result in a minimal amount of cell breakage; analyses of chromosome number frequently are influenced by the inclusion of cells that have lost chromosomes during preparation. Because of this potentially confounding factor, it is essential that hyperploidy and hypoploidy be recorded separately. A greater emphasis generally is placed on the values for hyperploidy as these are less likely to be influenced by technical artifacts.

The number of animals to be treated and cells to be analyzed is a matter of laboratory preference, although a general recommendation is for analysis of 100 metaphase II cells per animal or 100 first cleavage cells per treatment group (since the latter cell type is relatively difficult to obtain). The number of animals per treatment group, in part, will be determined by the statistical power desired.

In many assays for aneuploidy, it is important also to analyze chromosome structural aberrations. Such analysis will aid in the determination that the agent reaches the cells of interest and will provide information on its mutagenicity. Other chromosome abnormalities (e.g., polyploidy) should be noted but should not be used as an assessment of aneuploidy.

Development and application of more standardized protocols than those represented in the data base considered for this report are required. This is particularly the case for the analysis of first cleavage divisions, where very little work has been carried out. It is technically simple to prepare and analyze metaphase II cells; however, evidence of transmitted aneuploidy can be obtained from first cleavage studies. In addition, the male and female pronuclei are distinguishable at the first cleavage division and can be analyzed separately. In the case of treatment of the male, the male nucleus is analyzed for possible effects of the test chemical, with the female nucleus serving as one form of control and vice versa for treated females.

There is a need for determining the potential of the sperm decondensation test as a specific modification of first cleavage analysis. It is essential to develop technical improvements that will increase the number of analyzable cells and to confirm aneuploidy frequencies as true measures of induced or transmitted aneuploidy in man. Further development and validation of the numerical sex chromosome anomaly test for measuring aneuploidy is also needed.

New methods are being developed to increase both the accuracy and the speed of analysis for measuring aneuploidy in male germ cells. One such method involves the use of fluorochrome-labeled chromosome-specific DNA sequences that can be hybridized to DNA in sperm, other germ cells, or even somatic cells. The presence of more or less than one fluorescent spot in haploid cells or other than two fluorescent spots in diploid cells would be an indicator of aneuploidy.

A method under development that may prove informative regarding mechanisms of aneuploidy induction is that of synaptonemal complex analysis (Moses, 1986; Poorman et al., 1984, 1985). Several chemicals recently have been shown to interfere with normal chromosome pairing during meiotic prophase. Ultrastructure studies presently under way are aimed at determining whether or not various induced structural and behavioral perturbations to the synaptonemal complex correlate with aneuploidy measured in metaphase II cells. The practical utility of synaptonemal complex analysis as a sensitive assay for routine use in measuring mutagen-induced damage to mammalian germ cells is also being evaluated.

As discussed in this report, studies for determining the potential of chemical agents to induce aneuploidy in mammalian germ cells are of considerable importance. The current lack of data needs to be remedied, with special attention given to the development and use of reliable protocols.

References

Adler, I.-D. (1982) Mouse spermatogonia and spermatocyte sensitivity to chemical mutagens, Cytogenet. Cell Genet., 33, 95–100.

Adler, I.-D., A. Schmaltz, R. Rathenberg, D. Muller, F.F. Stasser and R. Perret (1978) Cytogenetic studies in sper-

matocytes of mice and Chinese hamsters after treatment with isoniazid (INH), Hum. Genet., 42, 50–54.

Albanese, R. (1982) The use of fertilized mouse eggs in detecting potential clastogens, Mutation Res., 97, 315–326.

Alvarez, M.R. (1983) Numerical chromosome variation in mouse spermatogonia resulting from alcohol consumption, J. Hered., 74, 58.

Au, W.W., and T.C. Hsu (1980) The genotoxic effects of adriamycin in somatic and germinal cells of the mouse, Mutation Res., 79, 351–361.

Badr, F.M., S.A. Rabouh and R. Badr (1979) On the mutagenicity of methadone hydrochloride, Induced dominant lethal mutation and spermatocyte chromosomal aberrations in treated males, Mutation Res., 68, 235–249.

Barlow, P. and C.G. Vasa (1970) The Y chromosome in human spermatozoa, Nature (London), 226, 961–962.

Binkert, F., and W. Schmid (1977) Preimplantation embryos of Chinese hamster, II. Incidence and type of karyotype anomalies after treatment of the paternal post-meiotic germ cells with an alkylating mutagen, Mutation Res., 46, 77–86.

Bond, D.J., and A.C. Chandley (1983) Aneuploidy, Oxford Monographs on Medical Genetics, Oxford University Press, New York.

Brandriff, B., L. Gordon, L. Ashworth, G. Watchmaker, A. Carrano and A. Wyrobek (1984) Chromosomal abnormalities in human sperm: comparisons among four healthy men, Hum. Genet., 66, 193–201.

Brook, J.D. (1982) The effects of 4CMB on germ cells of the mouse, Mutation Res., 100, 305–308.

Bulsiewicz, H. (1977) Influences of phenol on chromosomes of mice (*Mus musculus*) in the process of spermatogenesis, Folia Morphol. (Warsaw), 36(1), 13–22.

Bulsiewicz, H., and H. Wesolowski (1977) The mutagenic influence of sulfur on meiotic chromosomes in guinea pigs, Folia Morphol. (Warsaw), 36(1), 5–11.

Bulsiewicz, H., L. Rozewicka, H. Januszewska and J. Bajko (1976) Aberrations of meiotic chromosomes induced in mice with insecticides, Folia Morphol. (Warsaw), 35(3), 361–368.

Caspersson, T., L. Zech and C. Johansson (1970) Differential binding of alkylating fluorochromes in human chromosomes, Exp. Cell Res., 60, 315–319.

Cattanach, B.M., D. Papworth and M. Kirk (1984) Genetic tests for autosomal non-disjunction and chromosome loss in mice, Mutation Res., 126, 189–204.

Chandley, A.C., and R.M. Speed (1979) Testing for non-disjunction in the mouse, Environ. Health Perspect., 31, 123–129.

Dalterio, S., F. Badr, A. Bartke and D. Mayfield (1982) Cannabinoids in male mice: effects on fertility and spermatogenesis, Science, 216, 315–316.

De Boer, P., and F.A. Van Der Hoevern (1980) The use of translocation-derived 'marker-bivalents' for studying the origin of meiotic instability in female mice, Cytogenet. Cell Genet., 26, 49–58.

Dellarco, V.L., K.H. Mavournin and M.D. Waters (1986) A U.S. Environmental Protection Agency Special Committee Report: An evaluation of testing approaches for the detection of chemically induced aneuploidy, Mutation Res., 167, 3–7.

Devi, K.R., and P.P. Reddy (1980) Evaluation of thiotepa for genetic damage in mice, Ind. J. Exp. Biol., 18, 866–867.

Ficsor, G., H.V. Malling, H.W. Michelmann, D.B. Feldman and S.M. Fry (1979) Rhesus monkey (*Macaca mulatta*) model in genetic toxicology: mitomycin C clastogenicity in germ cells, Mutation Res., 59, 37–43.

Fontignie-Houbrechts, N. (1981) Genetic effects of formaldehyde in the mouse, Mutation Res., 88, 109–114.

Ford, J.H., and C. Roberts (1983) Displacement of chromosomes in mitosis: a technique for assessing differential chromosomal error, Cytogenet. Cell Genet., 36, 537–541.

Fowler, A.J., D.N. Singh and C. Dwivedi (1982) Effects of cadmium on meiosis. Bull. Environ. Contam. Toxicol., 29, 412–415.

Goetz, P., R.J. Sram and Z. Zudova (1974) The mutagenic effect of lysergic acid diethylamide, I. Cytogenetic analysis, Mutation Res., 26, 513–516.

Goetz, P., A.M. Malashenko and N.I. Surkova (1980a) Cyclophosphamide-induced chromosomal aberrations in meiotic cells of male mice, Folia Biol. (Prague), 26, 289–297.

Goetz, P., A.M. Malashenko and N.I. Surkova (1980b) Chromosome aberrations induced by cyclophosphamide in meiotic cells of male mice, Tsitol. Genet., 14(4), 29–35 (CYGED, 14, 27–32).

Gollapudi, B.B., O.P. Kamra and S.R. Blecher (1981) A search for a genetic basis for gonosomal univalency in mice, Cytogenet. Cell Genet., 29, 241–249.

Grell, R.F. (1959) Non-random assortment of non-homologous chromosomes in *Drosophila melanogaster*, Genetics, 44, 421–435.

Hansmann, I. (1983) Factors and mechanisms involved in nondisjunction and X-chromosome loss, in: A.A. Sandberg (Ed.), Cytogenetics of the Mammalian X Chromosome, Part A, Basic Mechanisms of X Chromosome Behavior, Liss, New York, pp. 131–170.

Henderson, S.A., and R.G. Edwards (1968) Chiasma frequency and maternal age in mammals, Nature (London), 218, 22–28.

Hook, E.B. (1983) ICPEMC Working Paper 5/3, Perspectives in mutation epidemiology, 3. Contribution of chromosome abnormalities to human morbidity and mortality and some comments upon surveillance of chromosome mutation rates, Mutation Res., 114, 389–423.

Kapp Jr., R.W., and C.B. Jacobson (1980) Analysis of human spermatogonia for Y chromosomal nondisjunction, Teratogen. Carcinogen. Mutagen., 1, 193–211.

Kapp Jr., R.W., D.J. Picciano and C.B. Jacobson (1979) Y-chromosomal nondisjunction in dibromochloropropane-exposed workmen, Mutation Res., 64, 47–51.

Klasen, M., and M. Schmid (1981) An improved method for Y-body identification and confirmation of a high incidence of YY sperm nuclei, Hum. Genet., 58, 156–161.

Martin, R.H., W. Balkan, K. Burns, A.W. Rademaker, C.C. Lin and N.L. Rudd (1983) The chromosome constitution of 1000 human spermatozoa, Hum. Genet., 63, 305–309.

Miltenburger, H.G., H. Trager, I.-D. Adler, A. Schmaltz, R. Rathenberg, H. Schwegler, I.H. Pawlowitzki, Ch. Nautsch and D. Muller (1978) The effects of isoniazid (INH) on Chinese hamster and mouse spermatogonia, Hum. Genet., 42, 44–49.

Mitra, J., D.M. Ca[illegible], A.S. Moorthy, J.L. Bue and T.N. Fredrickson (197[illegible]) Alterations in mitotic and meiotic chromosomes in BAL[illegible]B/c mice treated with LSD-25, Nucleus (Calcutta), 22(2), [illegible]0–87.

Moses, M.J. (1986) The synaptonemal complex in meiosis: significance of in[illegible]uced perturbations, this issue.

Nandan, S.D., and M[illegible]S. Rao (1982) Induction of chromosome aberrations by p[illegible]enobarbitone in the germ cells of Swiss male mice, Toxic[illegible]l. Lett., 14, 1–6.

Nehez, M., A. Selyp[illegible]s, A. Paldy and Gy. Berencsi (1978) The mutagenic effect [illegible]of a dinitro-*o*-cresol-containing pesticide on mice germ cel[illegible]s, Ecotox. Environ. Safety, 2, 401–405.

Norppa, H., T. West[illegible]rmarck, A. Oksanen, E. Rimaila-Parnanen and S. Knuutila (1980) Chromosomal effects of sodium selenite in vivo, I[illegible]Aberrations in mouse bone marrow and primary spermat[illegible]cytes, Hereditas, 93, 97–99.

Pacchierotti, F., A. Russo, R. Nave and P. Mettali (1982) Enhanced nond[illegible]junction frequency and decreased offspring productio[illegible]in a mouse colony treated orally with the antihelminthic d[illegible]g thiabendazole, Atti Assoc. Genet. Ital., 28, 293–295.

Pacchierotti, F., D. Bellincampi and D. Civitareale (1983) Cytogenetic obse[illegible]vation, in mouse secondary spermatocytes, on numeric[illegible]l and structural chromosome aberrations induced by cyclop[illegible]hosphamide in various stages of spermatogenesis, Mutati[illegible]n Res., 119, 177–183.

Pawlowitzki, I.H., [illegible]nd P.L. Pearson (1972) Chromosomal aneuploidy in h[illegible]uman spermatozoa, Hum. Genet., 16, 119–122.

Pecevski, J., N. Savk[illegible]ovic, D. Radiviojevic and L. Vuksanovic (1981) Effect of [illegible]l of nutmeg on the fertility and induction of meiotic chrom[illegible]osome rearrangements in mice and their first generation, [illegible]oxicol. Lett., 7, 239–243.

Pichan, K., and H. P[illegible]lasa (1982) Lack of effect of vitamin C on the incidence of chromosome anomalies induced by influenza virus in germ cells of mice, Hum. Genet., 61, 259–261.

Polani, P.E., and C[illegible]M. Jagiello (1976) Chiasmata, meiotic univalents, and a[illegible]ge in relation to aneuploid imbalance in mice, Cytogenet.[illegible]Cell Genet., 16, 505–529.

Poorman, P.A., J.V[illegible] Allen and M.J. Moses (1984) Synaptonemal compl[illegible]x analysis of mutagen-induced damage to mammalian germ[illegible]line chromosomes, Environ. Mutagen., 6, 404 (Abstr.).

Poorman, P.A., G.K[illegible] DeWeese, J.B. Gibson, J.W. Allen and M.J. Moses (198[illegible]) Synaptonemal complex analysis of alkylator induced [illegible]amage to rodent germ line chromosomes, Presented at the [illegible]Symposium on Aneuploidy: Etiology and Mechanisms, Ma[illegible]ch 25–29, Carnegie Institution, Washington, DC.

Rudak, E., P.A. Ja[illegible]obs and R. Yanagimachi (1978) Direct analysis of the c[illegible]romosome constitution of human spermatozoa, Nature (L[illegible]ndon) 274, 911–913.

Russell, L.B. (1976) Numerical sex-chromosome anomalies in mammals: their [illegible]pontaneous occurrence and use in mutagenesis studies, [illegible]n: A. Hollaender (Ed.), Chemical Mutagens, Vol. 4, Ple[illegible]um, New York, pp. 55–91.

Russo, A., F. Pacchi[illegible]rotti and P. Metalli (1984) Nondisjunction induced in mouse spermatogenesis by chloral hydrate, a metabolite of trichloroethylene, Environ. Mutagen., 6, 695–703.

Sankaranarayanan, K. (1982) Genetic Effects of Ionizing Radiation in Multicellular Eukaryotes and the Assessment of Genetic Radiation Hazards in Man, Elsevier Biomedical Press, Amsterdam.

Schmid, W. (1977) Remarks and data on some methods to monitor the in vivo induction of chromosome aberrations in mammals, in: A. de la Chapelle and M. Sorsa (Eds.), Chromosomes Today, Vol. 6, Elsevier/North-Holland Biomedical Press, Amsterdam, pp. 327–336.

Shiraishi, Y. (1978) Chromosome aberrations induced by monomeric acrylamide in bone marrow and germ cells of mice, Mutation Res., 57, 313–324.

Speed, R.M. (1977) The effects of aging on the meiotic chromosomes of male and female mice, Chromosoma, 64, 241–254.

Subramanyam, S., and Jameela (1977) Studies on cytological effects of frusemide on meiotic cells of male mice, Indian J. Med. Res., 66, 104–113.

Subramanyam, S., S. Ganeshan and P. Uma Devi (1981) Action of anti-allergic agent pheniramine maleate on mammalian somatic and meiotic chromosomes, Indian J. Exp. Biol., 19, 516–519.

Sugawara, S., and K. Mikamo (1983) Absence of correlation between univalent formation and meiotic nondisjunction in aged female Chinese hamsters, Cytogenet. Cell Genet., 35, 34–40.

Szemere, G. (1978) Male meiotic and post-meiotic studies: a new possible way of mutagenicity testing, Biol. Zentralbl., 97, 173–180.

Szemere, G., and I. Marczinovits (1977) EAAS and MMS-induced meiotic nondisjunction in the male mouse, in: D. Scott, B.A. Bridges and F.H. Sobels (Eds.), Progress in Genetic Toxicology, Elsevier/North-Holland Biomedical Press, Amsterdam, pp. 215–222.

Tates, A.D. (1979) *Microtus oeconomus* (Rodentia), a useful mammal for studying the induction of sex-chromosome nondisjunction and diploid gametes in male germ cells, Environ. Health Perspect., 31, 151–159.

Tates, A.D., and N. de Vogel (1981) Further studies on effects of X-irradiation on prespermatid stages of the Northern vole *Microtus oeconomus*, Low induction of sex-chromosomal nondisjunction and very high induction of diploid spermatids, Mutation Res., 82, 323–330.

Tates, A.D., P.L. Pearson and J.P.M. Geraerdts (1975) Identification of X and Y spermatozoa in the Northern vole *Microtus oeconomus*, J. Reprod. Fertil., 42, 195.

Tates, A.D., P.L. Pearson, M.v.d. Ploeg and N. de Vogel (1979) The induction of sex-chromosomal nondisjunction and diploid spermatids following X-irradiation of pre-spermatid stages in the Northern vole *Microtus oeconomus*, Mutation Res., 61, 87–101.

Van Buul, P.P.W., and J.H. Goudzwaard (1980) Bleomycin-induced structural chromosomal aberrations in spermatogonia and bone-marrow cells of mice, Mutation Res., 69, 319–324.

Wyrobek, A.J., G. Watchmaker, L. Gordon, K. Wong, D. Moore II and D. Whorton (1981) Sperm shape abnormalities in carbaryl-exposed employees, Environ. Health Perspect., 40, 255–265.

Wyrobek, A., G. Watchmaker, L. Gordon, A.V. Carrano, L. Ashworth and B. Brandriff (1984) Aneuploidy and quinacrine-positive spots in human sperm, Am. J. Hum. Genet., 36, 1185.

Yanagimachi, R. (1982) Potential methods for examining sperm chromosomes, in: R.P. Amann and G.E. Seidel (Eds.), Prospects for Sexing Mammalian Sperm, Colorado Assoc. Univ. Press, Boulder, CO, pp. 225–247.

NOTICE

This document has been reviewed in accordance with U.S. Environmental Protection Agency policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

This article documents research that was conducted under the auspices of the U.S. Environmental Protection Agency (USEPA). As an arm of the government, the USEPA has a paid up, non-exclusive, irrevocable, worldwide license to reproduce, prepare derivative works, and distribute to the public all of the material contained herein, and to grant others acting on its behalf the right to do so. The USEPA grants to the National Technical Information Service, Department of Commerce, the right to act as its agent in the distribution of this material.